

## **CALCIUM BLOCKERS TO TREAT PROLIFERATIVE RETINAL DISEASES**

### **CROSS REFERENCE TO RELATED APPLICATIONS**

5 This patent application is a continuation-in-part of U.S. Patent Application Serial  
No. 10/436,902, filed on May 12, 2003, which is a continuation of U.S. Patent  
Application Serial No. 10/038,215, filed on January 2, 2002, which is a  
continuation of U.S. Patent Application Serial No. 09/445,832 which was filed  
on December 13, 1999 as the U.S. National Patent Application of  
10 PCT/US98/12414, which was filed on June 15, 1998 and was based on U.S.  
Provisional Application 60/051,962, which was filed on June 30, 1997 in the  
name of Dreyer for CALCIUM BLOCKERS TO TREAT PROLIFERATIVE  
VITREORETINOPATHY. All of the aforementioned patent applications are  
expressly incorporated by reference herein.

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### **FIELD OF THE INVENTION**

This invention relates to the treatment of diseases related to the proliferation or  
migration of retinal pigment epithelium and/or glial cells.

20

### **BACKGROUND OF THE INVENTION**

Many diseases or conditions which threaten a person's vision are  
believed to be related to the migration or proliferation of retinal pigment  
25 epithelium and/or glial cells. Some examples of such diseases are non-  
exudative age related macular degeneration, exudative age related macular  
degeneration, choroidal neovascularization, acute macular neuroretinopathy,  
cystoid macular edema, diabetic macular edema, Behcet's disease, diabetic  
retinopathy, retinal arterial occlusive disease, central retinal vein occlusion,  
30 uveitic retinal disease, retinal detachment, trauma, conditions caused by laser  
treatment, conditions caused by photodynamic therapy, photocoagulation,  
radiation retinopathy, epiretinal membranes, proliferative diabetic retinopathy,

branch retinal vein occlusion, anterior ischemic optic neuropathy, non-retinopathy diabetic retinal dysfunction, and retinitis pigmentosa.

### **BRIEF DESCRIPTION OF THE INVENTION**

5           We have discovered that glutamate causes migration and proliferation of retinal pigment epithelium and/or glial cells. The use of glutamate antagonists to reduce or control retinal pigment epithelium and/or glial migration and the subsequent development of diseases or conditions is disclosed herein. Disclosed herein is a method of treating a disease or condition wherein  
10 migration or proliferation of retinal pigment epithelium or glial cells causes or contributes to the cause of said disease or condition, comprising administering a therapeutically effective amount of a compound which is a glutamate agonist to the patient suffering from said disease or condition.

### **DETAILED DESCRIPTION OF THE INVENTION**

          In relation to the methods of treating disclosed herein, the disease or condition being treated is a disease or condition wherein migration or proliferation of retinal pigment epithelium or glial cells causes or contributes to  
20 the cause of said disease or condition. The relationship may be direct or indirect, and the migration or proliferation retinal pigment epithelium or glial cells may be a root cause of said disease or condition, or may be a symptom of another underlying disease or condition. While not intending to limit the scope of the invention in any way, the following are examples of the types of diseases  
25 or conditions treated by the disclosed method: non-exudative age related macular degeneration, exudative age related macular degeneration, choroidal neovascularization, acute macular neuroretinopathy, cystoid macular edema, diabetic macular edema, Behcet's disease, diabetic retinopathy, retinal arterial occlusive disease, central retinal vein occlusion, uveitic retinal disease, retinal  
30 detachment, trauma, conditions caused by laser treatment, conditions caused by photodynamic therapy, photocoagulation, radiation retinopathy, epiretinal

membranes, proliferative diabetic retinopathy, branch retinal vein occlusion, anterior ischemic optic neuropathy, non-retinopathy diabetic retinal dysfunction, and retinitis pigmentosa.

In one method, disease or condition is selected from the group consisting  
5 of non-exudative age related macular degeneration, exudative age related macular degeneration, choroidal neovascularization, acute macular neuroretinopathy, cystoid macular edema, diabetic macular edema, Behcet's disease, diabetic retinopathy, retinal arterial occlusive disease, central retinal vein occlusion, uveitic retinal disease, retinal detachment, trauma, conditions  
10 caused by laser treatment, conditions caused by photodynamic therapy, photocoagulation, radiation retinopathy, epiretinal membranes, branch retinal vein occlusion, anterior ischemic optic neuropathy, non-retinopathy diabetic retinal dysfunction, and retinitis pigmentosa.

In another embodiment the disease or condition is not proliferative  
15 vitreoretinopathy.

In another method, the disease is proliferative diabetic retinopathy.

While not desiring to be bound to any specific theory, we conclude that one or more of the several types of calcium-permeable CNS ion channels mentioned below can be involved in controlling such migration, including: a)  
20 the various aspects of the NMDA (N-methyl-D-aspartate) receptor channel complex; b) the voltage-dependent  $\text{Ca}_{\text{v}}2+$  channels; and c) other channels directly coupled to glutamate (or excitatory amino acid) receptors. Such channels are reviewed in: Sommer, B. and Seeburg, P. H. "Glutamate receptor channels: novel properties and new clones" Trends Pharmacological Sciences  
25 13:291-296 (1992); Nakanishi, S., "Molecular Diversity of glutamate receptors and implications for brain function", Science 248:597-603 (1992).

The compound may be one of the so-called NMDA antagonists--i.e., it reduces neuronal damage mediated by the NMDA receptor complex. Alternatively, the compound antagonizes neuronal damage mediated by the  
30 voltage-dependent calcium channel. Other useful compounds are those which limit release of glutamate from cells or reduce the intracellular neurotoxic

consequences of glutamate interaction with cell membrane glutamate receptors. Preferably, the compound crosses the blood-retinal barrier.

Particularly preferred compounds are antagonists of the NMDA receptor-channel complex. The term "NMDA receptor antagonists" includes several sub-types of NMDA antagonists including: a) channel blockers--i.e., antagonists that operate uncompetitively to block the NMDA receptor channel; b) receptor antagonists--antagonists that compete with NMDA to act at the NMDA binding site; c) agents acting at either the glycine co-agonist site or any of several modulation sites such as the zinc site, the magnesium site, the redox modulatory site, or the polyamine site; d) agents which inhibit the downstream effects of NMDA receptor stimulation, such as agents that inhibit activation of protein kinase C activation by NMDA stimulation, antioxidants, and agents that decrease phosphatidylinositol metabolism.

Other compounds that are useful in the invention include voltage-dependent calcium channel antagonists, e.g. those which exert a substantial direct effect on glutamate toxicity mediated by the L-type voltage dependent  $\text{Ca}_{\text{v}}2.2$  channel in that they produce a statistically significant result in experiments measuring glutamate induced effects by the general method described in Karschian and Lipton, J. Physiol. 418:379-396 (1989) or by other techniques for measuring antagonism of the L-type  $\text{Ca}_{\text{v}}2.2$  channel known to those in the art. (We contrast the direct effect so measured with the secondary effects of excitotoxicity mediated by other channels, which in turn causes flow through the voltage dependent  $\text{Ca}_{\text{v}}2.2$  channels.) Particular candidate compounds include Class I voltage dependent  $\text{Ca}_{\text{v}}2.2$  channel antagonists, e.g., phenylalkylamines.

Preferably, the compounds used cross the blood-retina barrier and can be administered chronically. Other useful agents act as antagonists of non-NMDA receptors (glutamate receptor types other than the NMDA receptor complex discussed above), and include agents which block ionotropic glutamate receptors

or interact with metabotropic glutamate receptors (Nakanishi, supra). Still other agents act to limit (reduce) release of glutamate from cells, thereby acting upstream from the glutamate receptors in the excitatory neurotoxicity process. Still other agents may act by blocking downstream effects of glutamate receptor stimulation, e.g., the intracellular consequences of glutamate interaction with a cell membrane glutamate receptor, such as agents (like dantrolene) that block the rise in intracellular calcium following stimulation of membrane glutamate receptors.

The most preferred compounds are those capable of crossing the blood-retinal barrier; these compounds may be administered orally, intravenously, or topically and cross intervening barriers including the blood-retina barrier to reach the retinal ganglion cells. Compounds that do not freely cross the blood-retina barrier are less preferred; these compounds may be administered intravitreally to the retina. In the case of compounds that have an intermediate ability to cross the blood-retina barrier, the mode of administration will depend on the dosage required and other factors.

Among the preferred compounds are amantadine derivatives (e.g., memantine, amantadine, and rimantadine), nitroglycerin, dextorphan, dextromethorphan, and CGS-19755. See generally, the compounds listed in Table 2.

The invention is useful for the reduction or prevention (including prophylactic treatment) of damage as a result of proliferative vitreoretinopathy.

In view of our discovery that glutamate is associated with proliferative vitreoretinopathy, the invention features antagonists having certain specific characteristics: the ability to cross the blood-retina barrier; and the ability to be administered chronically. Within those guidelines, any suitable antagonist of the glutamate induced excitotoxicity may be used in accordance with the invention. As mentioned, in preferred embodiments, N-methyl-D-aspartate (NMDA) subtype of glutamate receptor-channel complex may be used to reduce or prevent proliferative vitreoretinopathy-related injury. Many antagonists of the

NMDA receptor have been identified (Watkins et al., Trends in Pharmacological Sci. 11:25, 1990, hereby incorporated by reference). There are several recognized sub-types of NMDA receptor including: a) channel blockers--i.e., antagonists that operate non-competitively to block the NMDA receptor channel; b) receptor antagonists--antagonists that compete with NMDA, acting at the NMDA binding site; c) agents acting at either the glycine co-agonist site or any of several modulation sites such as the zinc site, the magnesium site, the redox modulatory site, or the polyamine site; d) agents which inhibit the downstream effects of NMDA receptor stimulation such as agents that inhibit activation of protein kinase C activation by NMDA stimulation, antioxidants, and agents that decrease phosphatidylinositol metabolism.

Other compounds that are useful in this invention include non-NMDA receptor antagonists, such as agents which block other types of inotropic glutamate receptors or interact with metabotropic glutamate receptors; voltage-dependent calcium channel antagonists (against L, N, T, and P type channels) (Bean, B. P. Annu. Rev. Physiol. 51:367-384 (1989); Hess, P. Annu. Rev. Neurosci. 13:337-356 (1990)), and are described in greater detail below; and agents which act to decrease the release of glutamate, thereby acting upstream in the excitatory neurotoxicity process.

Table 1, below, lists various suitable NMDA and non-NMDA receptors which do not operate via the voltage-dependent  $\text{Ca}_{\text{sup.++}}$  ion channel. Tables 2-4 list antagonists of the voltage dependent  $\text{Ca}_{\text{sup.++}}$  channel, which can be used by themselves in connection with the first aspect of the invention, and which can also be used in combination with other antagonists in the second aspect of the invention.

NMDA Antagonists		NMDA Antagonists		NMDA Antagonists	
1.	Competitive NMDA Antagonists (act at agonist binding site) CGS-19755	2.	Channel Blockers (Un-Competitive NMDA Antagonists) MK-801	3.	Antagonists at Glycine Site of the NMDA Receptor Kyourenate, 7-

17322 CON3-CIP (AP)  
PATENT

7

5	acid	(CIBA- GEIGY) and other piperdine derivatives, D-2-amino-5- phospho- valerate, D-2-amino-7- phosphonohep- tanoate (AP7) CPP {[3-(2- carboxy-	(Dizocilpine) and other derivatives of dibenzy- ocycloheptene (Merck)	chloro- kyourenate, 5,7-chloro- kyourenate, thio- derivatives, and other derivatives. (Merck)
10			Sigma receptor ligands, e.g.	Indole-2- carboxylic
15		piperazin-4-y- propyl-1-phos- phonic acid]]	Dextrorphan, dextro- methorphan and morphinan derivatives (Hoffman La Roche) such as cara- miphen and timeazole (which also block calcium channels)	
20				
25				
30		LY27614, CGP39551, CGP37849, LY233053, LY233536	Ketamine, Tiletamine and other cyclo- hexanes	DNQX
35		O-phospho- bornoserine	Phencyclidine (PCP) and derivatives, and pyrazine	Quinoxaline or oxidiazole derivatives including
	CNQX,		compounds	NMQX

	MDL100,453	Memantine,	Glycine
	partial		
5		amantadine, rimanta- dine and derivatives	agonist (e.g. Hoecht-Roussel P-9939)
10		CNS 1102 (and related bi- and tri- substituted guanidines)	
15		Diamines Conantokan peptide from Cocus geographus Agatoxin-489	
20	4. Polyamine Site of NMDA Receptor	5. Redox Site of NMDA Receptor	6. Other Non- Competitive NMDA Antagonists
25	Arcaïne and related biguani- dines and biogenic polyamines Ifenprodil and Carvedilol	Oxidized and reduced glutathione  PQQ (pyrrolo-	Hoechst 831917189  SKB
30	related drugs Diethylene- triamine SL 82.0715	quinoline) Compounds that generate Nitric Oxide (NO) or other oxi- dation states of nitrogen monoxide (NO+, NO-) including those listed in the	
35			

	box below
	Nitroglycerin
	and
	derivative,
5	Sodium Nitro-
	prusside, and
	other NO
	generating
	listed on p. 5
10	of this table
	Nitric oxide
	sythase (NOS)
	Inhibitors:
	Arginine
15	analogs
	including N-
	mono-methyl-
	L-argine
	(NMA):
20	N-amino-L-
	arginine
	(NAA);
	N-nitro-L-
	(NNA);
25	N-nitro-L-
	arginine methyl
	ester; N-imino-
	ethyl-L-
	ornithine
30	Flavin
	Inhibitors:
	diphenyl-
	iodinium;
	Calmodulin
35	inhibitors,
	trifluoperizine
	Calcineurin
	Inhibitors, e.g.,
	FK-506

10

(inhibits  
calcineurin  
and thus NOS  
diphos-  
phorylase)

5

	Inhibitors of Downstream Effects of NMDA	Inhibitors of Downstream Effects of NMDA	Non-NMDA Receptor Antagonists
	7. Agents to inhibit protein kinase C activation by NMDA stimu- lation (involved in NMDA toxicity) MDL 27.266 (Merrill Dow) and triazole- one derivatives Monosialo- gangliosides (eg GM1 of Fidia Corp.) methoxylisox- and other gang- lioside derivatives LIGA20, LIGA4 (may also effect calcium extrusion via calcium ATPase)	8. Downstream effects from Receptor Activation  8a. To decrease phopshati- dylinositol metabolism kappa opioid receptor agonist: U50488  (Upjohn) and dynorphan  kappa opioid receptor agonist:	9A. Non-NMDA antagonists (Competitive)  CNQX, NBQX, YM900, DNQX, PD 140532  AMOA (2-amino- 3[3-9carboxy- methoxyl-5- azol-4-yl] propionate)  2-phospho- phonoethyl phenylamine
10			
15			
20			
25			
30			
35			

11

PD117302, derivatives,

i.e.

CI-977 5-ethyl, 5-

methyl,

5

5-

trifluoromethyl

8b. To decrease  
hydrogen  
peroxide and  
free radical  
injury, eg  
antioxidants

10

21-  
aminosteroid

9B. Non-NMDA  
Non

15 competitive

(lazaroids) antagonists  
such as

U74500A,

U75412E and

20

U74006F

U74389F, GYK152466

FLE26749,

Trolex (water  
soluble alpha

25

tocophenol),  
3,5-dialkoxy-4-  
hydroxy-

benzylamines

Compounds Evans Blue

30

that generate

Nitric Oxide

(NO) or

other oxidation

states of

35

nitrogen

monoxide

(NO+, NO-)

including

those listed in

12

the box below  
Nitroglycerin  
and  
derivatives,  
Sodium Nitro-  
prusside, and  
other NO  
generating  
listed on p. 5  
of this  
table  
Nitric oxide  
synthase (NOS)  
Inhibitors:  
Arginine  
analogs in-  
cluding N-  
mono-methyl-  
L-arginine  
(NMA); N-  
amino-L-  
arginine  
(NAA); N-  
nitro-L-  
arginine  
(NNA); N-  
nitro-L-  
arginine methyl  
ester, N-  
iminoethyl-L-  
ornithine

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25

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35

Agents Active at  
Metabotropic

Glutamate

Receptors

10a. Blockers of  
Metabotropic  
Glutamate

Decrease

glutamate release

11. Agents to  
decrease  
glutamate

Drugs to decrease  
intracellular calcium  
following glutamate  
receptor stimulation

12a. Agents to  
decrease  
intracellular

	Receptors	release	calcium
release			
5	AP3 (2-amino-3-phosphonoprionic acid)	Adenosine, and derivatives, e.g. cyclohexyladenosine	Dantrolene (sodium dantrium); Ryanodine (or ryanodine + caffiene)
10b.			
10			
	Agonists of	CNS1145	12b. Agents
15	Metabotropic Glutamate Receptors		inhibiting intracellular Calcium-ATPase
20	(1S,3R)-1-Amino-cyclopentane-1,3-dicarboxylic acid [(1S,3R)-1,4-	Conopeptides: SNX-111, SNX-183, SNX-230	Thapsigargin, cyclopiazonic acid, BHQ ([2,5-di-(tert butyl)-
25	ACPD], commonly ref as `trans`-ACPD		benzohydroquinone; 2,5-di-(tert butyl)-1,4 benzohydroquinone])
30		Omega-Age-IVA, toxin from venom of funnel web spider	
35		Compounds that generate Nitric Oxide (NO) or other oxidation states of nitrogen	

14

5 monoxide  
(NO+, NO-)  
including  
those listed  
in the box  
below  
Nitroglycerin  
and  
10 derivatives,  
Sodium Nitro-  
prusside, and  
other NO  
generating  
15 listed on p. 5  
of this table  
Nitric oxide  
synthase (NOS)  
Inhibitors:  
Arginine  
20 analogs  
including N-  
mono-methyl-  
L-arginine  
(NMA);  
25 N-amino-L-  
arginine (NAA)  
N-nitro-L-  
arginine  
(NNA);  
30 N-nitro-L-  
arginine methyl  
ester;  
N-iminoethyl-  
L-ornithine  
35 Additional NO-  
generating  
compounds  
Isosorbide  
dinitrate

15

(isordil)

S-nitrosocapto-  
pril (SnoCap)

Serum albumin

5

coupled to  
nitric oxide  
(SA-NO)

Cathepsin

10

coupled to  
nitric oxide  
(cathepsin-NO)

Tissue

plasminogen

activator

15

coupled to  
NO (TPA-NO)  
SIN-1 (also  
known as SIN1

or molsi-

20

domine)  
Ion-nitrosyl  
complexes  
(e.g.,

nitrosyl-iron

25

complexes,  
with iron in the  
Fe<sup>2+</sup> state)

Nicorandil

30

TABLE 2

Antagonists of the Voltage Dependent Calcium Channels

(N, L, T, P and other types)

dihydropyridines

35

(e.g., nimodipine)

phenylalkylamines

(e.g., verapamil, (S)-emopamil, D-600, D-888)

benzothiazepines

(e.g., diltiazem and others)

5 bepridil and related drugs  
diphenylbutylpiperdines  
diphenylpiperazines  
(e.g., flunarizine/cinnarizine series)  
HOE 166 and related drugs  
fluspirilene and related drugs  
toxins and natural compounds  
(e.g., snail toxins -  
.omega.conotoxin GVIA and GVIIA, maitotoxin,  
10 taicatoxin, tetrandine, hololena toxin, plectreury's  
toxin, funnel-web spider venom and its toxin fraction,  
agatoxins including .omega.-agatoxin IIIA and .omega.-  
agatoxin IVA.

15

TABLE 2

Antagonists of the Voltage Dependent Calcium Channels  
(N, L, T, P and other types)

20 dihydropyridines  
(e.g., nimodipine)  
phenylalkylamines  
(e.g., verapamil, (S)-emopamil, D-600, D-888)  
benzothiazepines  
(e.g., diltiazem and others)  
25 bepridil and related drugs  
diphenylbutylpiperdines  
diphenylpiperazines  
(e.g., flunarizine/cinnarizine series)  
HOE 166 and related drugs  
30 fluspirilene and related drugs  
toxins and natural compounds  
(e.g., snail toxins -  
.omega.conotoxin GVIA and GVIIA, maitotoxin,  
taicatoxin, tetrandine, hololena toxin, plectreury's  
35 toxin, funnel-web spider venom and its toxin fraction,  
agatoxins including .omega.-agatoxin IIIA and .omega.-  
agatoxin IVA.

TABLE 4

## OTHER CALCIUM CHANNEL ANTAGONISTS

5	diclofurime	D-600
	pimozide	D-888
	prenylamine	Smith Kline 9512
	fendiline	ranolazine
	perhexiline	lidoflazine
10	mioflazine	CERM-11956
	flunarizine/	R-58735
	cinnarizine series	R-56865
	verapamil	amiloride
	dilfiazine	phenytoin
15	dipropervine	thioridazine
	(S)-emopamil	tricyclic antidepressants

## In Vitro Assay

20

An antagonist may be tested for utility in the method of the invention by monitoring its effect on proliferative retinopathy as follows.

25 Cultured fibroblasts will be injected into the vitreous of the rabbit eye. After two weeks, the degree of vitreopathy can be assessed histologically. At the time of the initial insult, the animals will be treated with the compound under consideration.

Such models are well known. A few examples (hereby incorporated by  
30 reference) included Kiumura et al. Human Gene Therapy, 7:799-808 (1996); Sakamoto et al., Ophthalmology 102:1417-1421 (1995); Handa et al. Experimental Eye Research 62:689-696 (1996); Berger et al. 37:2318-1325 (1996); de Souza et al. Ophthalmologica 209:212-216 (1995); Nakagawa et al. Ophthalmology & Visual Science 36:2388-2395 (1995); Steinhorst et al.  
35 Archive for Clinical & Experimental Ophthalmology 232:347-354 (1994).

## Use

An effective receptor antagonist will cause a decrease in proliferative  
5 vitreoretinopathy. As described above, the preferred compounds which cross the  
blood-retinal barriers are preferably administered topically or orally in known,  
physiologically acceptable vehicles including tablets, liquid excipients and  
suspensions. Those skilled in the art will appreciate how to formulate acceptable  
therapeutics.

10

Antagonists may be compounded into a pharmaceutical preparation, using  
pharmaceutical compounds well-known in the art; the exact formulation and  
dosage of the antagonist compound depends upon the route of administration.  
Generally, the effective daily dose of the antagonists will range from 0.01 to  
15 1000 mg/kg.

## Other Embodiments

Other embodiments are within the following claims. In the method of the  
20 invention, a useful compound may be administered by any means that allows  
the compound access to the retina. The compounds useful in the method include  
antagonists of excitatory amino acid receptors (both NMDA and non-NMDA  
subtypes) that act to reduce retinal cell migration or proliferation or reduce  
binding of glutamate to the NMDA receptor. The antagonists can act at a  
25 modulatory site or a co-agonist site or by blocking the chain of events initiated  
by receptor activation.